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(58) Field of search C2C
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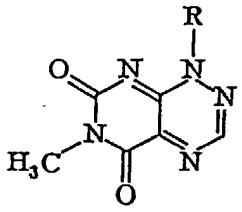
(54) Preparation of reumycin

(57) Reumycin, 6-methylpyrimidino [5,4-e]-*as*-triazine-5, 7-(6*H*8*H*)-dione which has anti-tumour activity may be prepared by reacting a compound of formula



(in which R represents a lower alkyl group, e.g. methyl) with an amine, preferably a primary or secondary amine or an amino group-containing anionite and then acidifying the reaction product. The intermediate produced in this reaction is a quaternary ammonium salt of reumycin, which is also new.

ERRATUM

SPECIFICATION NO 2039883A

Page 5, line 63, *after* amine. *Start new paragraph insert* New claims or amendments to claims filed on 1st May 1980. Superseded claims 5 New or amended claims:-

Original claims 6 to 9 renumbered as 5 to 8 respectively.

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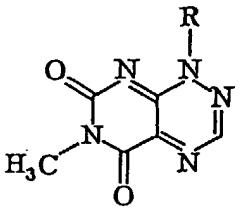
(54) **Preparation of reumycin**

(57) Reumycin, 6-methylpyrimidino [5,4-e]-*as*-triazine-5, 7-(6*H*8*H*)-dione which has anti-tumour activity may be prepared by reacting a compound of formula



(in which R represents a lower alkyl group, e.g. methyl) with an amine, preferably a primary or secondary amine or an amino group-containing anionite and then acidifying the reaction product. The intermediate produced in this reaction is a quaternary ammonium salt of reumycin, which is also new.

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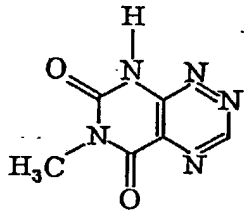
SPECIFICATION

Preparation of Reumycin

The present invention relates to the preparation of the antibiotic reumycin and to certain novel intermediates used in this preparation.

Reumycin, 6-methylpyrimidino[5.4-e]-*as*-triazine-5,7(6*H*,-8*H*)-dione, has the formula:

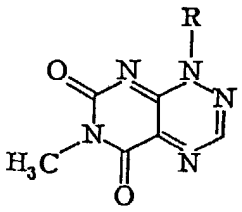


(I)

It has recently become of considerable interest, since it has shown anti-tumour activity and there is, therefore, a need for a method of preparing reumycin in good yield and purity.

We have now discovered that reumycin may be prepared by the dealkylation of a pyrimidinotriazine derivative by reaction with an amine, followed by treatment with an acid.

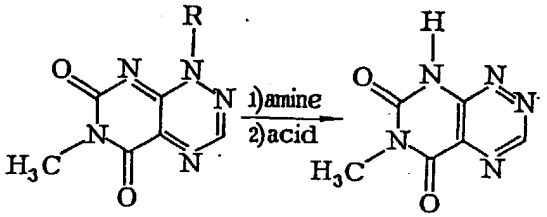
Thus, the present invention consists in a method of preparing reumycin, which comprises reacting a pyrimidino-triazine derivative of formula II):



(II)

(in which R represents a lower alkyl group) with an amine and then acidifying the reaction product.

The method of the invention can be represented by the following reaction scheme:



The pyrimidinotriazine derivatives of formula (II) employed in the method of the invention include known compounds, such as xanthothricin (toxoflavin) in which R represents a methyl group, as well as new compounds, which can be prepared by methods similar to those described above [cf J. Amer. Chem. Soc., *83*, 3904-3905 (1961)].

Xanthothricin can be prepared by culturing certain micro-organisms on suitable nutrient media. Examples of such microorganisms include *Streptomyces brunneus* subspecies *xanthothricini*, as described in our co-pending British Application No. , or *Pseudomonas cocovenenans*. The fermentation broth containing xanthothricin can be used directly in the process of the invention or crude xanthothricin can be separated from the fermentation broth before reaction.

The amines employed in the method of the invention are preferably primary or secondary amines, examples of which include: aliphatic amines, such as mono- or di- alkylamines (in which the alkyl group may be, for example, one or more of methyl, ethyl, propyl or butyl); alkenylamines, such as allylamine; aralkylamines, such as benzylamine or dibenzylamine; and cyclic amines, such as piperidine, piperazine, pyrrolidine or morpholine. It is also possible to use as the amine a weakly basic anionite containing kprimary and/or secondary amino groups, for example Amberlite IR45(OH^-), a trade name for a product obtainable from Rohm and Haas Co.), or anionite AH22(OH^-) (a trade name for a product obtainable from the USSR Ministry of Chemical Industry, being a weakly basic polymerized anionite having a polystyrene matrix and containing primary and secondary amino groups). We prefer that the amine used should have a pK_b value of from 2 to 6.

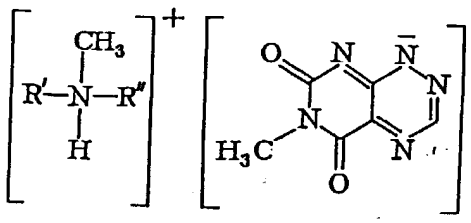
The method of the invention is preferably conducted in the presence of a solvent. Suitable solvents include: alcohols, for example methanol, ethanol or propanol; ketones, for example acetone or methyl ethyl

ketone; water; and mixtures of any two or more thereof.

Dealkylation reactions are, in general, carried out at alkaline pH values (preferably within a pH range of from 8 to 12), which normally necessitates the presence of a base such as an alkali metal or ammonium hydroxide. Since, however, the amines employed in the method of the invention are basic, it is normally unnecessary to employ any additional base.

The reaction is preferably carried out at a temperature within the range from 10 to 30°C, normally at about room temperature.

The reaction of xanthothricin with an amine results in the formation of a quaternary ammonium salt of formula (III):



(III)

(in which N H R'R'' represents an amine). These quaternary ammonium salts are novel compounds and they and their preparation also form part of the present invention.

To produce reumycin, the reaction mixture obtained from the reaction of the pyrimidinotriazine derivative (II) with the amine is treated with an acid. The nature of the acid is not critical and either organic acids (e.g. formic acid or acetic acid) or inorganic acids (e.g. hydrochloric acid or sulphuric acid) may be used. However, before the acid treatment, we prefer to concentrate the reaction mixture and, for this purpose, we prefer to remove some or all of the solvent by evaporation under reduced pressure.

The reumycin obtained by the method of the present invention may then, if desired, be purified by conventional techniques, for example by recrystallization from a suitable solvent, e.g. ethanol.

The reumycin obtained by the method of the invention comprises yellow crystals melting at 242 - 243°C (from ethanol). It has an Rf value on thin layer chromatography on silica gel developed with a 3 : 2 mixture of ethyl acetate and benzene of 0.50 and the following other physical properties:

Ultraviolet spectrum (ethanol(λ_{max} nm (log ε):
235(4.25);
265 shoulder (3.49);
340 (3.72);
400 (2.87)

Infrared absorption spectrum KBr γ cm^{-1}:
3400, 3170, 3110, 3080, 3020, 2995, 2950, 2900,
2870, 2815, 1738, 1700, 1670, 1685, 1630, 1590,
1480, 1445, 1429, 1380, 1842, 1295, 1270, 1258,
1213, 1180, 1130, 1070, 1065, 970, 943, 930,
840, 830, 770, 750, 730, 716, 700, 690, 580,
560, 470.

Proton magnetic resonance spectrum (pyridine D_5, TMS) δ ppm:
3.39 (3H, singlet);
9.84 (1H, singlet);
10.94 (1H, enlarged singlet).

Solubility (per 100 ml of solvent):

water	2.04 g;
physiological solution	2.00 g;
ethanol	0.55 g.

Elemental analysis:
Found: C, 40.9%; H, 2.7%; N, 39.1%.
Calculated for $C_6H_5N_5O_2$ (179.14):
C, 40.2%; H, 2.8%; N, 39.1%.

In contrast to other antibiotics of the pyrimidino[5. 4-e]-*as*-triazine group, reumycin prepared by the method of the present invention possesses substantially lower toxicity, which makes it possible to administer it to the human organism in concentrations ensuring a pronounced anti-tumour effect without any intoxication caused by the compound.

Tests for biological activity of reumycin prepared by the method of the present invention have shown clearly pronounced activity against mice tumours in the solid form, specifically against carcinoma 755, Harding-Passey melanoma, Fischer lymphadenosis in its ascitic form and Ehrlich carcinoma, Data illustrating the anti-tumour activity of reumycin are given in the following Table 1.

TABLE 1

Dose, mg/kg	Administration route	Tumour growth inhibition, %			
		Ehrlich carcinoma	Fischer lymphadenosis	Carcinoma 755	Harding-Passey melanoma
2.5-3.5	Intravenous	47-57.0	55.0	47-51	45.0
1.5-2.5	Intraperitoneal	41.0	65-66	58-61	54.0-56.0
50-75.0	*Per os*	30.0	45.0	47-51	55-60

The reumycin was administered repeatedly over a period of 9 days and, as can be seen from the Table displayed significant activity in the following doses (mg/kg):

2.5 - 3.5 intravenously;
1.5 - 1.5 intraperitoneally; and
50 - 75 *per os*.

Growth inhibition of carcinoma 755 and Harding-Passey melanoma on administration of reumycin intravenously, intraperitoneally and *per os* varied within the range from 45 to 61%. Thus under the conditions of the therapeutic tests, the anti-tumour activity of reumycin has been established for all routes of administration.

Reumycin exerts no detrimental effect on the growth or development of young animals. No allergic reaction is observed upon intracutaneous injection of reumycin to guinea pigs three weeks after sensitization, followed by a single injection of the compound.

The reumycin prepared by the method of the invention can be used in traditional pharmaceutical forms combined, if desired, with various pharmaceutically acceptable carriers or adjuvants. Examples of formulations in which the reumycin may be administered include, for the oral route, capsules, granules, powders, tablets and pills, as well as suppositories or injections. Carriers which may be used for the preparation, of pharmaceutical compositions include lactose, glucose, starch, cellulose, methyl cellulose, carboxymethylcellulose, talc, sodium citrate, calcium carbonate, magnesium stearate and distilled water.

The dose of reumycin administered will vary depending upon the route of administration and other factors, including the age and body weight of the patient and the nature of the tumour. In general, we prefer to administer from 100 to 150 mg per day orally, from 20 to 40 mg per day by intravenous or intramuscular injection, or from 40 to 60 mg per day by subcutaneous injection.

The method of the invention is illustrated by the following Examples.

Example 1

500 mg (2.59 mmole) of xanthothricin were dissolved in 30 ml of a 70% aqueous solution of diethylamine and the mixture was left for 30 - 40 minutes at room temperature. At the end of this time, the solvent was evaporated off *in vacuo*. The residue was acidified with dilute acetic acid and the mixture was again evaporated. The resulting reumycin was purified by recrystallization from ethanol. The yield was 451 mg (97% of theory). The product had a melting point of 242 - 243°C.

Ultraviolet spectrum (ethanol) λ_{max} nm (logε):

235 (4.25);
265 shoulder (3.49);
340 (3.72);
400 (2.87).

Example 2

500 mg of xanthothricin were treated under the same conditions as described in Example 1, but using a 1.70% aqueous solution of monoethylamine in place of the diethylamine. The yield of reumycin was 394 mg (85%), melting point 242 - 243°C.

Example 3

Amberlite IR45(OH^-) was added in small portions to a solution of 260 mg (1.35 mmole) of xanthothricin in 150 ml of water at a pH value of 8 - 9 until the yellow colour disappeared; this required a total of 350 ml. The

anionite was then washed with 250 ml of distilled water and then reumycin was eluted from it with dilute acetic acid. The eluate was evaporated and the reumycin left as a residue was purified by recrystallization from ethanol, to give 229 mg (95%) of a product melting at 242 - 243°C.

Example 4

Anionite AH22 (OH^-) was added in small portions to a solution of 193 mg (1 mmole) of xanthothricin in 60 ml of water at a pH value of 8.8 until the yellow colour had disappeared, which required a total of 560 ml. The mixture was then subsequently treated as described in Example 3, giving 152 mg (85%) of reumycin, melting point 242 - 243°C.

Example 5

An aqueous solution of xanthothricin (300 mg per 100 ml) was passed through a column (3 × 75 cm) packed with Amberlite IR45 (OH^-). The resin was washed with 1.5 litres of water and then eluted with 2.5 litres of 5% acetic acid. The fractions containing reumycin were combined and evaporated *in vacuo*. The residue was recrystallized from ethanol, yielding 250 mg (90%) of reumycin, melting point 242 - 243°C, Rf value 0.50 (silica gel, developed with 3 : 2 by volume ethyl acetate/benzene).

Example 6

An aqueous solution of xanthothricin (340 mg per 100 ml) was passed through a column (4 × 65 cm) packed with anionite AH22 (OH^-). Subsequent treatment was carried out following the procedure described in Example 5, to give 268 mg (85%) of reumycin, melting point 242 - 243°C, Rf value 0.50 (silica gel, developed with 3 : 3 by volume ethyl acetate/benzene).

Examples 7 - 17

These Examples illustrate the preparation of reumycin by the reaction of xanthothricin with a number of different amines.

To a solution of 193 mg (1 mmole) of xanthothricin in 15 ml of water were added 3 - 4 mmole of one of the amines shown in Table 2. The solution was stirred at room temperature for a period of from 30 to 190 minutes, depending upon the amine employed. The time taken for the yield of reumycin to reach a maximum was determined spectrophotometrically and the reaction time in each Example is shown in Table 2. The reaction mixture was then evaporated, the residue was acidified with acetic acid, the mixture was again evaporated and finally the product was recrystallized from an alcohol. The amines employed, reaction times and yields of product are given in Table 2.

TABLE 2

Example No.	Amine	Reaction time, min.	Yield of reumycin, %
7	methylamine	30	89.5
8	allylamine	75	73.2
9	benzylamine	90	85
10	dimethylamine	40	98
11	diethylamine	40	97.1
12	dibutylamine	170	97.5
13	dibenzylamine	190	75.0
14	piperidine	90	77.6
15	piperazine	120	84
16	pyrrolidine	75	93
17	morpholine	120	79.5

Example 18

410 mg (2.1 mmole) of xanthothricin were dissolved in 100 ml of acetone, and then 3.5 ml (21 mmole) of dibutylamine were added to the resulting solution. After 2.5 hours, the reaction mixture was acidified with 0.1N hydrochloric acid to a pH of 3 - 4 and the solvent was evaporated off. The residue was recrystallized from ethanol, giving 332 mg (87% of theory) of reumycin, melting point 242 - 243°C.

Example 19

200 mg (about 1 mmole) of xanthothricin were dissolved in 250 ml of absolute ethanol, and then 1.75 ml (10.5 mmole) of dibutylamine were added to the solution. The reaction mixture was subsequently treated as described in Example 18, to give 164 mg (88%) of reumycin, melting point 242 - 243°C.

Example 20

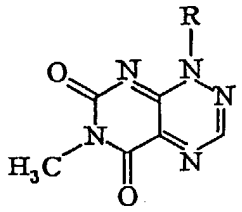
285 mg (1.5 mmole) of xanthothricin were dissolved in 50 ml of a 1 : 1 mixture of ethanol and water. 10 mg (12 mmole) of piperazine were then added to the solution and the reaction mixture was allowed to stand for 1.5 hours. At the end of this time, the mixture was acidified with 0.1N hydrochloric acid to a pH value of 3 - 4, the solvent was evaporated off and the residue was recrystallized from ethanol. The yield of reumycin was 225 mg (85%), melting point 242 - 243°C.

Example 21

The mycelium was separated from 6 litres of the culture broth of *Streptomyces brunneus* subspecies *xanthothricini*. The remaining solution (with an activity of 90 mcg/ml) was acidified with 20% sulphuric acid to a pH value of 5.8 - 6.0 and then extracted with chloroform. The extract was dried with anhydrous sodium sulphate and then evaporated at a temperature of 40°C. The residue was dissolved in 50 ml of distilled water, which had been made alkaline to a pH of 8.0 - 10.5. Anionite AH22 (OH^-) was then added until the colouration disappeared. The anionite was separated off and washed with distilled water and then reumycin was eluted from it with 5% acetic acid. After evaporation of the eluate and recrystallization, there were obtained 410 mg (76%) of reumycin, melting point 242 - 243°C.

CLAIMS

1. A method of preparing reumycin, which comprises reacting a pyrimidinotriazine derivative of formula (II):



(II)

(in which R represents a lower alkyl group) with an amine and acidifying the reaction product.

2. A method according to Claim 1, in which R represents a methyl group.

3. A method according to Claim 1 or Claim 2, in which said amine is a primary amine.

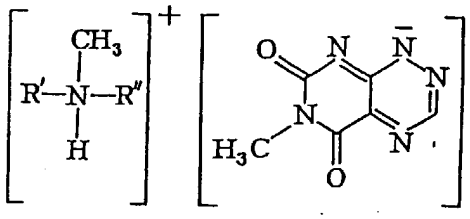
4. A method according to Claim 1 or Claim 2, in which said amine is a secondary amine.

5. A method according to Claim 1 or Claim 2, in which said amine is an anionite containing amino groups.

6. A method according to Claim 1, substantially as hereinbefore described with reference to any one of the foregoing Examples.

7. Reumycin when prepared by a method according to any one of the preceding Claims.

8. A compound of formula:



(in which N H R' R'' represents an amine).

9. A method of preparing a quaternary ammonium salt of reumycin, which comprises reacting xanthothricin with an amine.

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